Microscopic Anatomy

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Critical review

Human uterine cervix microvascularisation: application of corrosion casting and scanning electron microscopy

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Abstract

Introduction

The aim of this critical review is to summarise the knowledge on the subject of the entire microvascular architecture of the human uterine cervix and the way the specimens should be prepared for corrosion casting and further examinations using scanning electron microscopy.

Conclusion

In the authors opinion, corrosion casting combined with scanning electron microscopy assessment is currently the best available technique to visualise vascular architecture.

Introduction

The growth, development and regression of blood vessels are all key features of reproduction. The vascular system of the uterus has a unique remodelling capability associated with the menstrual cycle, implantation and pregnancy[1]. It is for this reason that the study of uterine microvascularisation spans over several centuries using a variety of techniques[2].

The uterine cervix receives most of its blood supply from the uterine artery[3]. The classic approach divides the course of the uterine artery into three parts—the descending, transverse and ascending. Lateral branches originate mostly from the transverse and ascending parts. Among those branches are also vessels supplying the uterine cervix[3]. Palacios Jaraquemada et al.[4] reported that blood is supplied to the cervix via the uterine artery, the cervical arteries (in 67% originating from the uterine artery) and the vaginal arteries (originating from the uterine artery or the internal iliac artery).

The microvascular architecture of the human uterus is well studied[5–8], but the majority of the relevant publications deals with the vasculature of the uterine corpus. In contrast, there exists a very limited number of studies on the subject of the microvascular architecture of the human uterine cervix[9,10]. This study primarily describes the vessels of the ectocervical mucosa. Studies dealing with the microvascularisation of the endocervical mucosa are even more rare[11–13].

The vasculature of the female reproductive organ is especially interesting for clinicians. This is because of surgical interventions undertaken to stop bleeding that may occur in the course of cervical cancer, ectopic pregnancies or leiomyomata.

The aim of this critical review is to summarise the knowledge on the subject of the entire microvascular architecture of the human uterine cervix and the way the specimens should be prepared for corrosion casting and further examinations using scanning electron microscopy (SEM).

Discussion

The authors have referenced some of their own studies in this review. These referenced studies have been conducted in accordance with the Declaration of Helsinki (1964) and the protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed. All human subjects, in these referenced studies, gave informed consent to participate in these studies.

A historical overview of preparing injections for corrosion casting of uterine microvasculature

The idea of anatomical modelling of the internal structures of organs dates back to the Middle Ages by the way of corrosion casting[2]. The injection technique was first proposed and used by Mondino de Luzzi (Mundinus) (circa 1291) to aid in his anatomical teaching[2].

The uterine injection studies were prepared in the following manner. Firstly, warm water was injected into veins. This was done for two reasons—to warm the anatomical parts, and to wash out the blood. Care was taken that all the water was removed before injecting the mass. Secondly, the mass was injected. The art of creating anatomical models was sometimes considered 'holy'[14].

The injectable mass had to be fluid in one state, and solid in the other. The first one occurred due to heat and the latter due to cold. The injectable material would consist of wax, resin, turpentine varnish and tallow, in proportions according to the kind of preparation. Once cooled, attempts to bend it were made—if it broke, then it was too hard. If it bent very easily, it was too soft. Ideally, it did not to bend without some force[2,14].

For the specimens to appear 'proper' to the eye, colour had to be added to the mass. The colours that were generally used were vermillion, king's yellow, blue verditer and flake white[2].

The time frame between the injection of the vessels and placing the specimens into an acid bath needed

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to be after the injection mass had taken a solid form. After the soft tissues had sufficiently corroded, the cast would be cleaned by pouring water on it[14].

Using the classic injection technique, John Hunter conducted a study (circa 1754) to establish the independence of the maternal and foetal circulations in the placenta[15].

Daron[16] in 1936 provided one of the most important works on the subject of uterine microvasculature (in rhesus monkeys), with detailed illustrations of endometrial microvasculature, using intravascular colloidal mercuric sulphide injections. These observations were the first detailed descriptions of the full-length of the endometrial arteries.

In 1938, Holmgren[17] delivered important practical information stating that the injection of uteri should ideally be performed directly after hysterectomy or otherwise rigor mortis of the arterial musculature and the myometrium would decrease the adequacy of injection. A major improvement regarding the injection material arrived in 1944 when Faulkner[18] utilised synthetic liquid latex (Neoprene). This technique produced clear and predictable arterial and venous casts of the uterine vasculature, however, added little to the existing body of knowledge[2,18].

In 1981 Rogers and Gannon[19] used corrosion casting and SEM to provide a method that was simple, rapid and reliable. It allowed, for the first time, for the three-dimensional visualisation of rat uterine vasculature. This method provided a clear picture of vessel interconnections, as well as demonstrating the vessel diameter and spacing at the time of casting. It also allowed for tracking changes in endometrial vasculature throughout the various stages of the menstrual cycle.

Further improvements in corrosion casting and SEM brought imaging to a new level.

Authors technique for preparing uterine cervix microvasculature corrosion casts for SEM assessment

All of the pharmaceutical products and equipment given in this section are the ones, the authors of this manuscript used to prepare the specimens during their studies.

Firstly, during excision, the uterus together with the ovaries and the cervical portion of the vagina has to be removed in such a way that relatively long fragments of uterine and ovarian vessels (both arteries and veins) are retained.

Immediately after removal, the uterus has to be perfused via the afferent arteries with prewarmed (37°C), heparinised saline (Heparin, Polfa, Poland, 12 IU mL^{-1}) containing 3% dextrane (70 kDa) and 0.025% lidocaine (Lignocaine, Polfa), until the fluid outflowing via the veins will be completely transparent (about 5–10 min)[20,21]. Next, perfusion has to be continued using a solution of 0.66% paraformaldehyde/0.08% glutaraldehyde (Sigma) in 0.1 M cacodylate buffer (pH 7.4) supplemented with 0.2% lidocaine. Finally, the vascular system has to be injected with 60–80 mL of Mercox CL-2R resin (Vilene Comp. Ltd., Japan) containing 0.0625 mg mL^{-1} methyl methacrylate with the polymerisation initiator (Vilene Comp. Ltd.)[22,23]. Then the uterus has to be left in a warm water bath (56°C) for 12 hours to allow for the polymerisation and tempering of the resin[24]. After completion of polymerisation, the tissues of the uterus have to be macerated for 5–6 days by repeated soaking in 10% potassium hydroxide at 37°C, followed by washing with warm (50°C–55°C) running tap water. Next, the obtained vascular casts have to be washed in several changes of distilled water, cleaned in 5% trichloroacetic acid for 1–2 days, washed again in distilled water for 2–3 days and freeze-dried in a lyophiliser (Liovag G2, Aqua Fina, Germany). Later, parts of the freeze-dried casts corresponding to the uterine cervix and a small fragment of the uterine body have to be excised and examined macroscopically. To facilitate sectioning of the casts, it is good to embed them at 55°C in a mixture of polyethylene glycols (PEG 2000/PEG 600, 20:1), next cool them to room temperature to solidify PEG[25] and gently dissect the casts longitudinally in the plane of the endocervical canal to expose the vasculature of the cervical wall. The sectioned fragments have to be washed with stirred distilled water to remove PEG and stored in an exsiccator containing phosphorus pentoxide until microscopic examination. Next, the fragments have to be mounted onto copper plates using colloidal silver and 'conductive bridges'[26] and coated with gold. We examined the casts using a JEOL SEM 35-CF scanning electron microscope at 20–25 kV. We distinguished the casts of arteries and arterioles from those of veins and venules on the basis of different imprints of endothelial cell nuclei[27].

Microvascular architecture of the human uterine cervix visualised using corrosion casting and SEM

When assessing blood supply to the cervix, we did not find in our studies a 'cervicovaginal' artery, which was reported to exist by Chen et al.[28] Most of the studies[3,4,11–13] show that the cervix is supplied by several vessels originating from the uterine artery on different levels. These vessels supply both the left and right parts of the cervix and divide into a series of smaller branches, which penetrate the paracervix. The smaller branches from the left and right side communicate by a series of anastomoses.

It is worth mentioning that no differences between multiparas and nulliparas can be noted in the vascular architecture of the human uterine cervix[11–13].

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Cervical supravaginal vascular architecture

Right below the serous membrane one can identify densely packed arteries and veins (diameter ~500 μm), which when going towards the canal of the cervix merge into a zone of large vessels (0.8–1 mm).

At the junction between the supravaginal part of the cervix and the corpus of the uterus, four distinct vascular zones can be distinguished (Figure 1). Going from lateral to medial (towards the cervix canal) one can identify the:

- outer zone containing large arteries and veins;
- zone of arterioles and venules of the muscular layer, characterised by loose and irregular texture;
- zone of endocervical mucosal capillaries, characterised by dense texture;
- pericanalar zone containing small veins and capillaries.

The zones of the cervix are a continuation of the uterine body wall layers. The zone of larger blood vessels corresponds to the vascular layer of the myometrium, whereas the other three zones belong to the mucosal layer[29].

Large arteries and veins from the outermost zone give off branches that run towards the canal of the cervix and supply or drain the mucosal capillaries. Along their course the branches divided and form capillaries (diameter 12–18 μm) which are arranged in a 'ladder' or 'H'-shaped fashion (Figure 2). Towards the vaginal portion of the cervix, the vessels assume a pattern of parallel capillaries running increasingly obliquely to the axis of the endocervical canal and showing relatively few interconnections.

In the subepithelial region of the cervical canal are situated veins (diameter 80–150 μm), which drain the mucosal capillaries. The veins run parallel to the long axis of the cervical canal and are joined by capillaries (diameter 12–20 μm) that run perpendicular to their course. The capillaries cover the veins, forming a sort of plexus around the larger vessels (Figure 3). The subepithelial veins can be observed along the whole length of the cervical canal with the exception of its last segment close to the external os. Their number, depending on canal segment, ranges from 1 to 4. This proves that the vasculature of the cervix is a part of the classic circulatory system.

The veins that we have described above might well suggest the existence of two drainage systems of the mucosal capillary plexus—one using venules and veins of the middle and peripheral zones, and the other based on small pericanalar veins. The veins of the second drainage system might contribute to the formation of pregnancy-induced cervical varices, which may lead to thrombosis[30].

Both in the muscular layer as well as in the pericanalar zone, there are numerous places where the arterioles

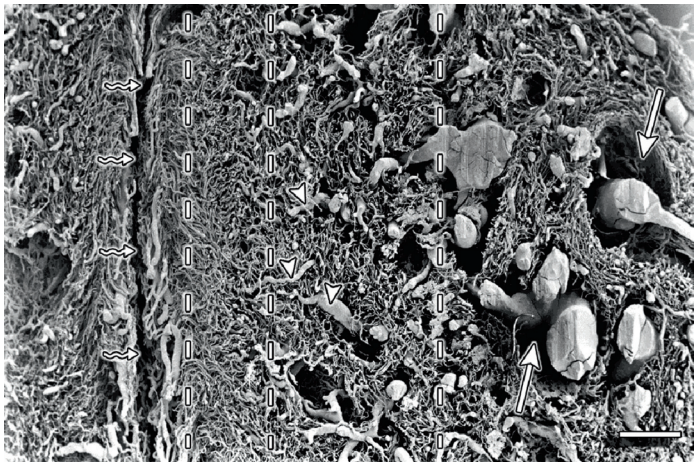

Figure 1: Cervix sagittal section—supravaginal part. Vertical interrupted lines divide the vasculature into four zones. The outermost zone is on the far right of the figure. The straight arrows point to the places where perivascular connective tissue was located. The triangles mark the vessels that run transversely and supply the pericanalar vessels. 'Serpent' arrows point to the cervical canal. Scale bar: 1000 μm[11].

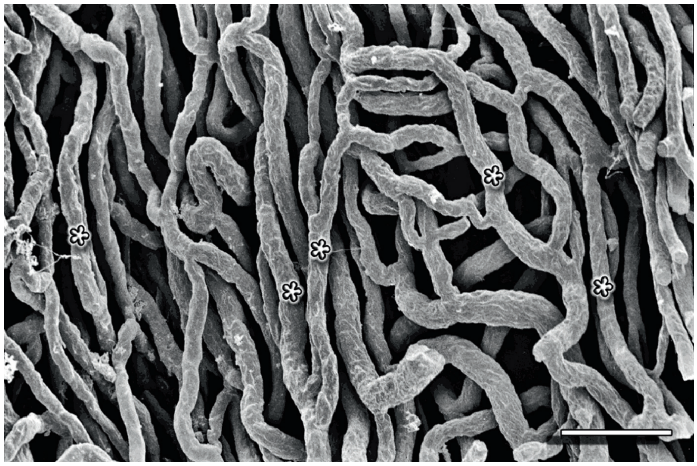

Figure 2: Cervix sagittal section—supravaginal part. Perivascular zone—capillaries arranged in a 'ladder' or 'H'-shaped fashion. The asterisks mark the capillaries forming the 'ladder'. Scale bar: 100 μm[11].

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and venules pass close to each other, often adjoining. This may well suggest that there exists a countercurrent transport between veins and arteries.

The cervical glands are surrounded by a dense capillary network (Figure 4). The plexuses are composed either of parallel capillaries (lower part of Figure 4) or irregularly formed vessels (upper part of Figure 4).

Cervical vaginal vascular architecture

Vessel arrangement in the vaginal part of the cervix is similar to the one described in the supravaginal part. However, the arteries and veins of the outer zone are of significantly smaller calibre and the veins of the pericanalar zone are much more exposed (Figure 5).

The vessels of the vaginal part of the cervix, near the external os of the uterus, form three separate layers:

- supplying vessels—characterised by relatively large diameters;
- oblique or perpendicular small arteries and veins;
- subepithelial capillaries joining the, above mentioned, oblique and perpendicular vessels.

Conclusion

Concluding, in the authors opinion, corrosion casting combined with SEM assessment is currently the best available technique to visualise vascular architecture. It offers high resolution and quasi-three-dimensional images of the vessels, without interference from the non-vascular tissue.

The cervix is supplied by several vessels originating from the uterine artery on different levels. These vessels supply both the left and right parts of the cervix and divide into a series of smaller branches, which penetrate the paracervix. The smaller branches from the left and right side communicate by a series of anastomoses.

No differences, between multiparas and nulliparas, can be noted in the vascular architecture of the human uterine cervix.

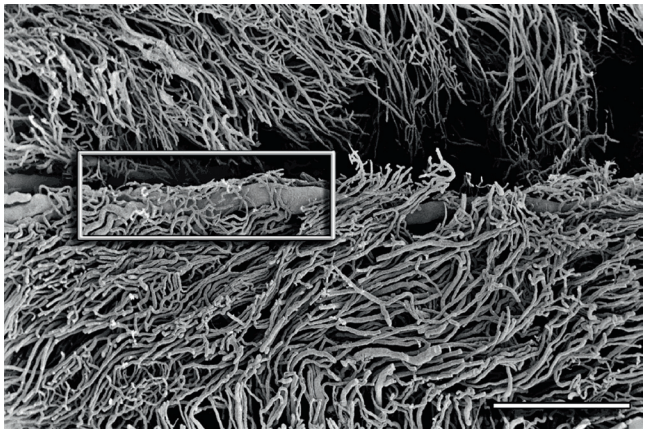

Figure 3: Cervix sagittal section—supravaginal part. Pericanalar vein covered by capillary plexus (marked by box). Scale bar: 1000 μm[11].

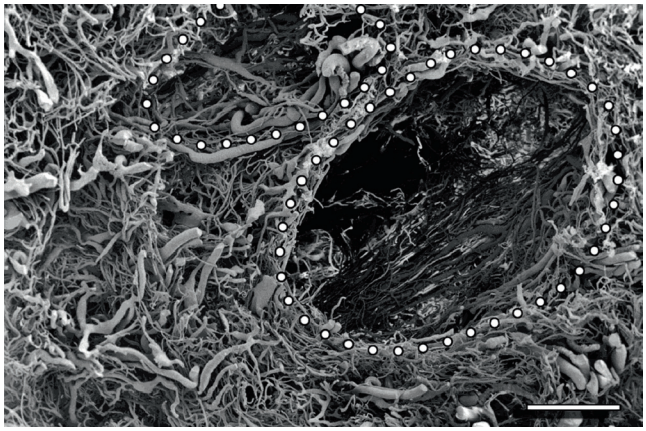

Figure 4: Cervix coronal section—supravaginal part. The dots mark the spaces where cervical glands were located. The glands were separated from each other by characteristically flattened veins. Scale bar: 1000 μm.

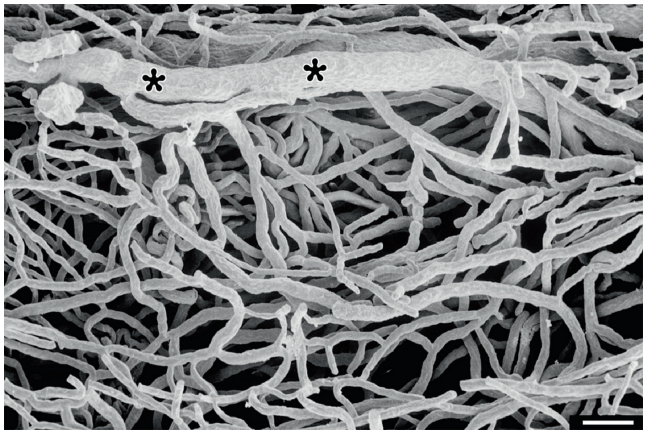

Figure 5: Cervix sagittal section—vaginal part. The asterisk mark the large, exposed, pericanalar veins. The capillaries join the pericanalar veins. Scale bar: 100 μm.

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Both in the vaginal and supravaginal parts of the human uterine cervix, four distinct vascular zones can be distinguished—the outer zone containing large arteries and veins, the arteriole and venule zone, the endocervical mucosal capillaries zone and the pericanalar zone containing small veins and capillaries. In the pericanalar zone run small veins, responsible for draining the mucosal capillaries. Both in the muscular layer, as well as in the pericanalar zone, arterioles and venules pass close to each other, often adjoining. This most probably confirms the existence of a countercurrent transport between adjoining veins and arteries.

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